Risk assessment of Salmonella in Danish meatballs produced in the catering sector

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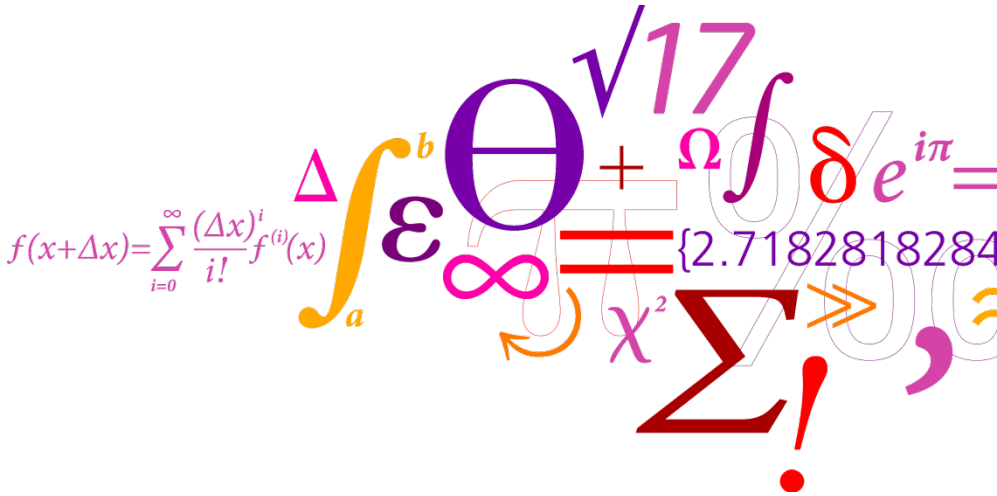


DTU Food
National Food Institute

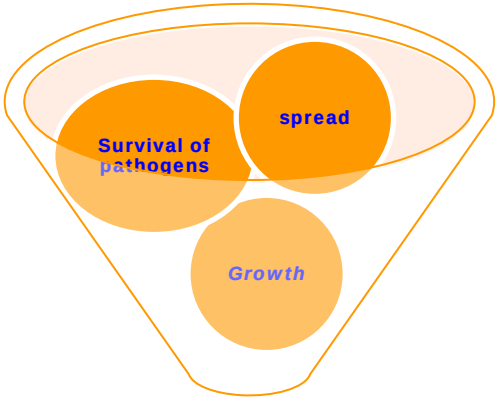
Outline:

- Introduction
- Objectives of the study
- Summarizing the performed work
- Process to build up the developed model
- Results
- Remarks and future perspectives

Introduction

- *Salmonella* is a critical pathogen (CDC, 2011; EFSA, 2010).
- Pork still is an important source of salmonellosis (EFSA, 2010; van Hoek *et al*., 2012; Wegener *et al*., 2003).
- Ground meat is frequently associated with outbreaks of salmonellosis (Stock and Stolle, 2001).
- Up to 70% of foodborne illnesses are estimated to be linked to catered food (Filion and Powell, 2011; Hensen et al., 2006; Jones et al., 2004; Lee and Middleton, 2003).
- In Denmark, 61 of 86 reported outbreaks in 2011 were associated with outside-the-home settings (anonymous, 2012).
- To model the distribution of pathogens during the processing operation are of major relevance to risk analysts (Flores, 2006).

Objectives of the study



Challenging Food safety → **Quantitative Microbiological Risk Assessment - QMRA**

risk of salmonelosis by consumption of “frikadeller”, processed by the catering sector

Based on scenario analysis:

- to evaluate existing practices
- challenge the efficiency of different Food Safety Authorities recommendations

Catering processing

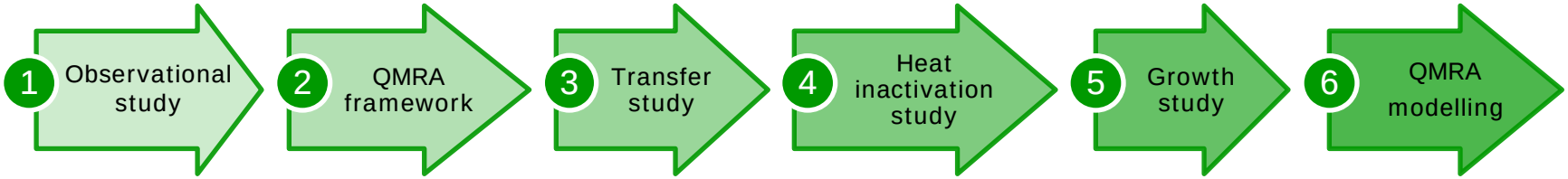
Raw pork

Final meal

Frikadelle

Summarizing the performed work

Experimental work and modelling activities were performed:



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ORIGINAL ARTICLE

Modelling transfer of *Salmonella* Typhimurium DT104 during simulation of grinding of pork

C.O.A. Møller[1], M.J. Nauta[1], B.B. Christensen[2], P. Dalgaard[3] and T.B. Hansen[1]

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Effect of natural microbiota on growth of *Salmonella* spp. in fresh pork – A predictive microbiology approach

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Food microbiology

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1 Observational study

Experimental work

School canteen

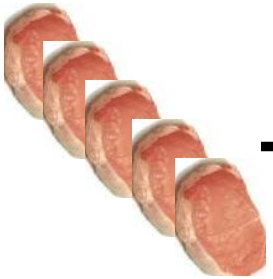
Object of investigation:
Danish catering sector

Worksite canteen

- Process flow
- Time and temperature profiles
- Weight of ingredients

- Unit changes during the processing, e.g.:

1 kg meat pieces → 200 g slices → 200 g portions → 5 kg batter → 70-80 g "*frikadelle*" batter


2 QMRA framework

Risk of salmonellosis from consumption of “frikadeller”

Modelling activities

Pathway

Processing step	Basic activity	Source/model reference
Reception	1. initial contamination	Hansen et al. (2010)
Slicing	2. partitioning	Nauta (2005)
Grinding slices into portions	3. cross contamination	Møller et al. (2012)
Mixing of ingredients	4. mixing	Nauta (2005)
Dividing into meatballs	5. partitioning	Nauta (2005)
Heating inactivation in pan	6. inactivation	this study
Holding time	7. growth	Møller et al. (2013), this study
plus		
Heat inactivation in oven	8. inactivation	this study
Serving time plus cold storage until 6° C is reached	7. growth	Møller et al. (2013), this study
Estimation of the risk	9. dose response	FAO/WHO (2002)

2 QMRA framework

Risk of salmonellosis from consumption of ‘frikadeller’

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Serving time plus cold storage until 6° C is reached	7. growth	Møller et al. (2013), this study
Estimation of the risk	9. dose response	FAO/WHO (2002)

2 QMRA framework

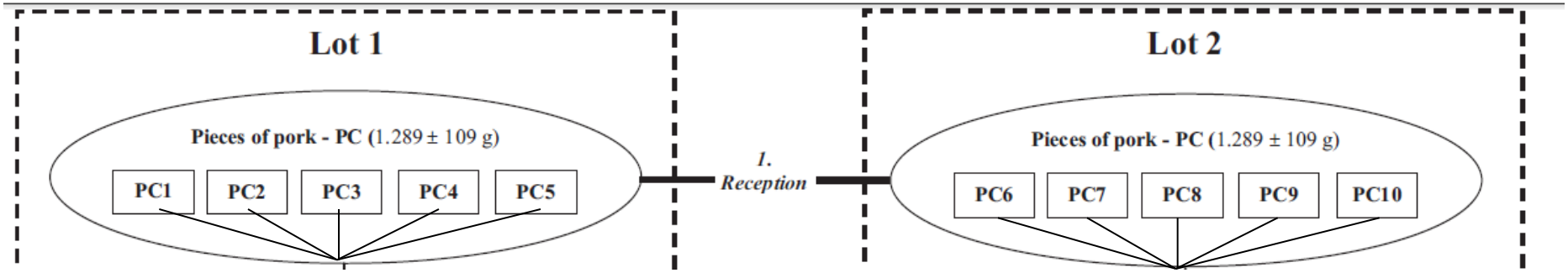
Risk of salmonellosis from consumption of 'frikadeller'

Processing step	Basic activity	Source/model reference
Reception	1. initial contamination	**Hansen et al. (2010)**

$R_{prevalence}$ = 4.2% of the samples

- 39 positive samples within the interval 0.04–0.4 CFU/g
- 7 positive samples within the interval 0.4–4 CFU/g
- 5 positive samples within the interval 4–40 CFU/g
- 1 positive sample with more than 40 CFU/g (assumed max. 400 CFU/g)

~Histograma (log(0.04), log(400), {39,7,5,1})

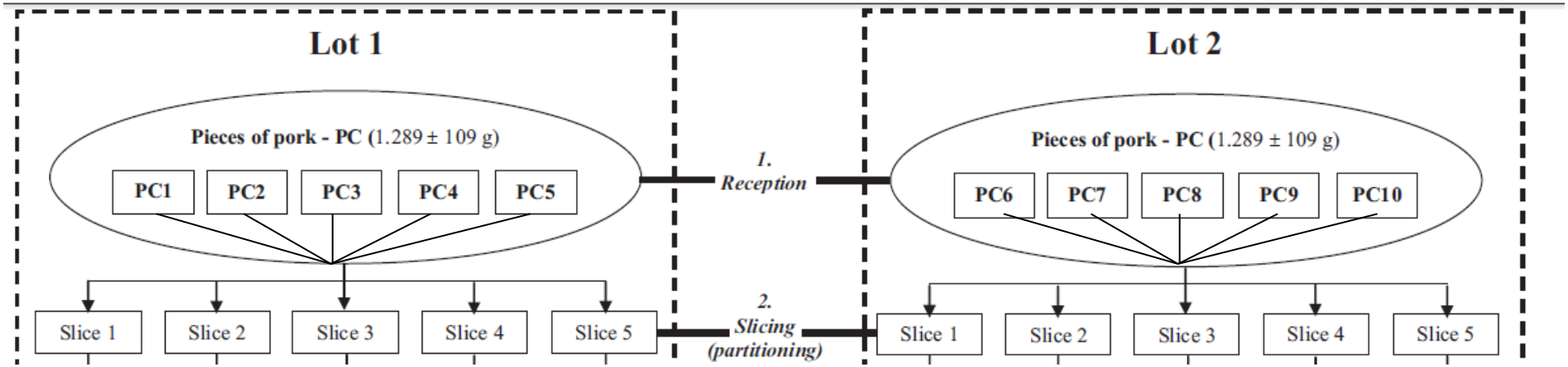


2 QMRA framework

Risk of salmonellosis from consumption of “frikadeller”

Processing step	Basic activity	Source/model reference
Reception	1. initial contamination	Hansen et al. (2010)
Slicing	2. partitioning	Nauta (2005)

$S_{conc\ slice} \sim Multinomial(Rconc\ piece, \{1/Sn\ slices\})$



2 QMRA framework

Risk of salmonellosis from consumption of “frikadeller”

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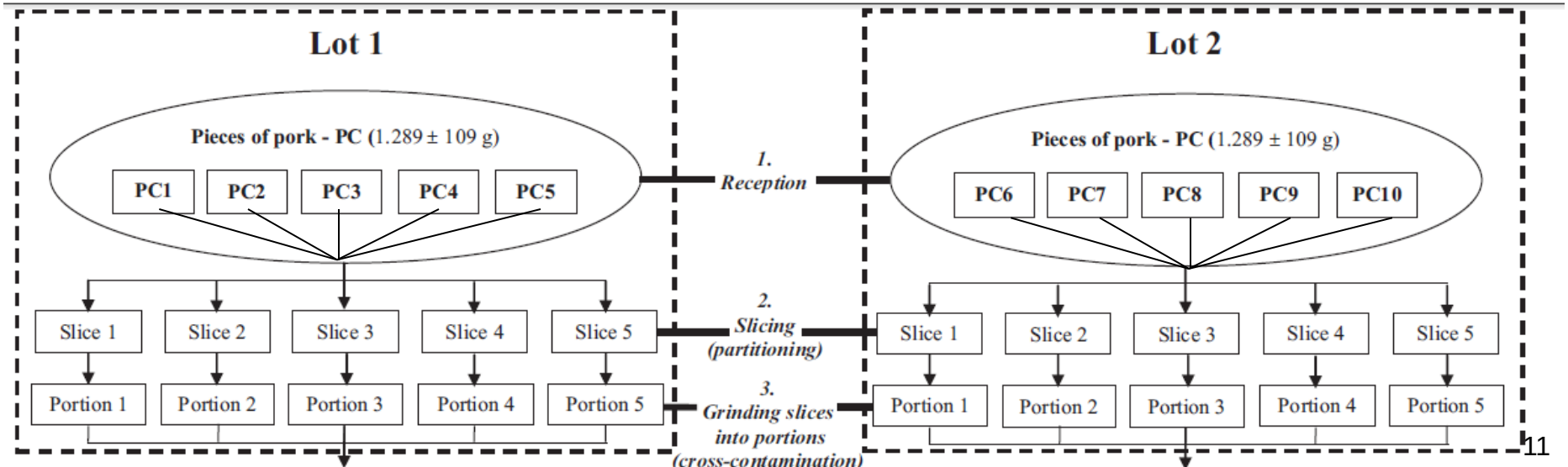
Grinding slices into portions	3. cross contamination	Møller et al. (2012)

$$M_i = (1-a_1)(1-a_2)(1-c_2)\, S_i + (b_1\, gr_{1,i-1}) + (b_2\, gr_{2,i-1})$$



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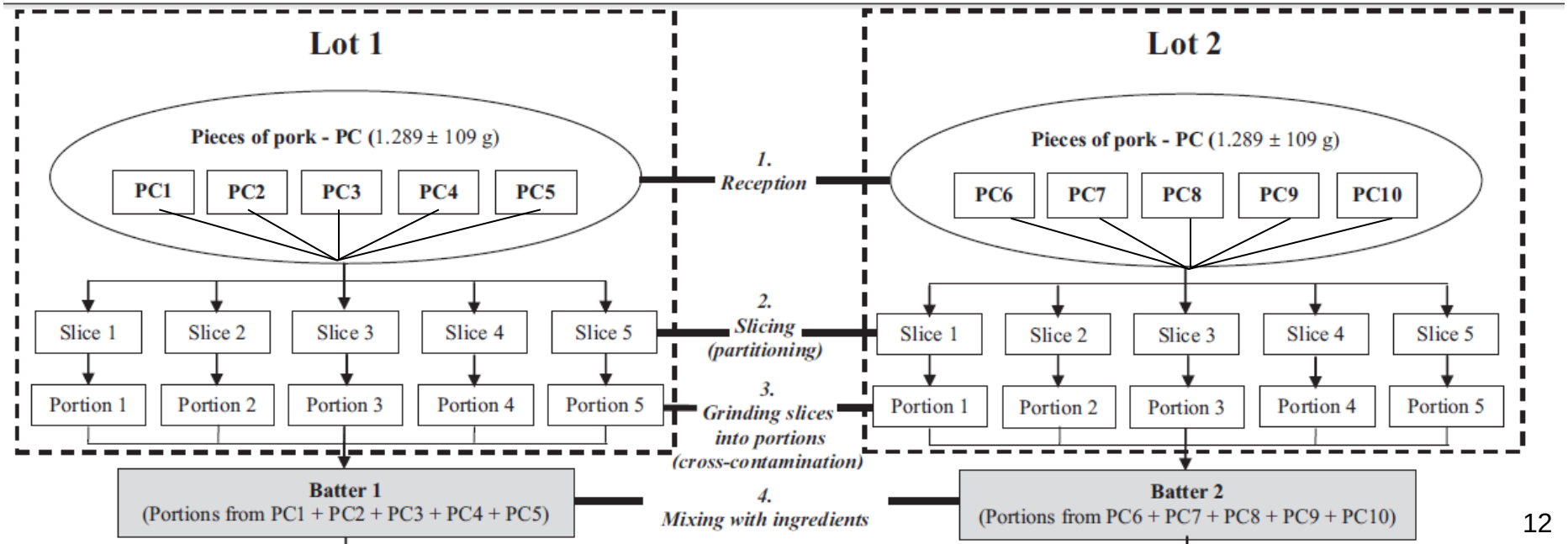
Grinding slices into portions	3. cross contamination	Møller et al. (2012)
Mixing of ingredients	4. mixing	Nauta (2005)

- the meat typically corresponds to 2/3 of the total weight,
- so the total weight is the weight of the meat multiplied by 3/2.



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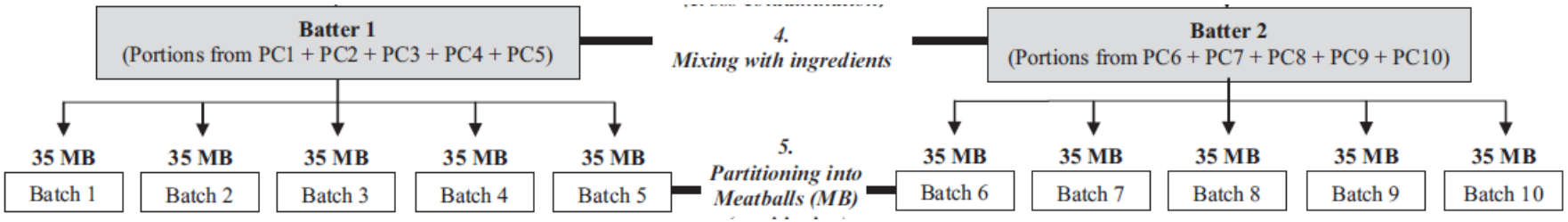
Mixing of ingredients	4. mixing	Nauta (2005)
Dividing into meatballs	5. partitioning	Nauta (2005)

$\sim Multinomial(M_{conc\ batter\ lot}, \{1/P_{weight\ Meatball\ of\ Pn\ MB\ samples}\})$



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Risk of salmonellosis from consumption of “frikadeller”

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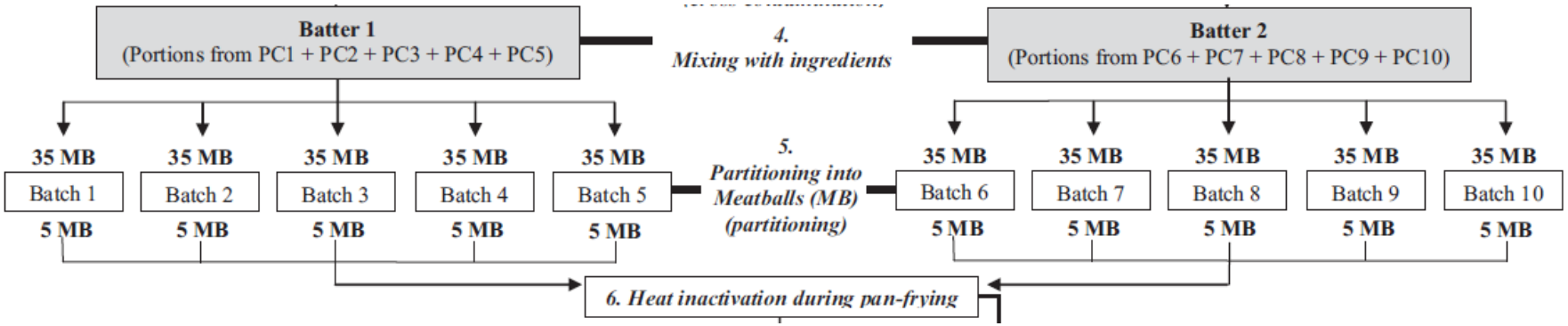
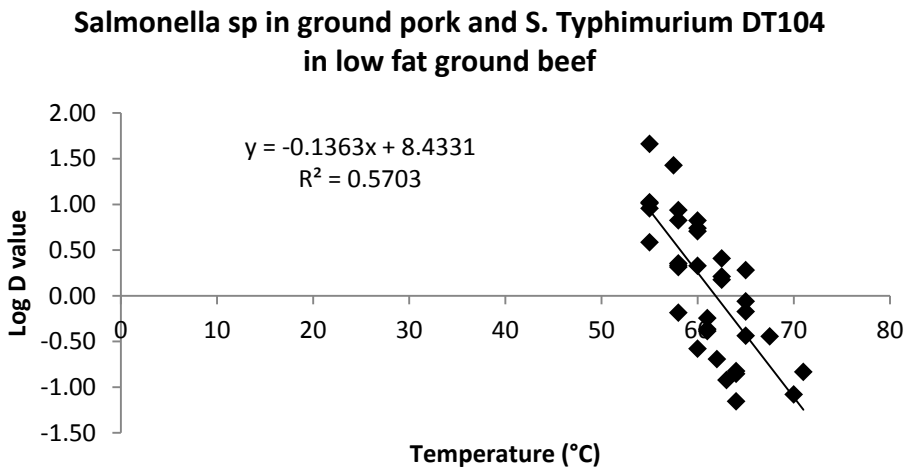
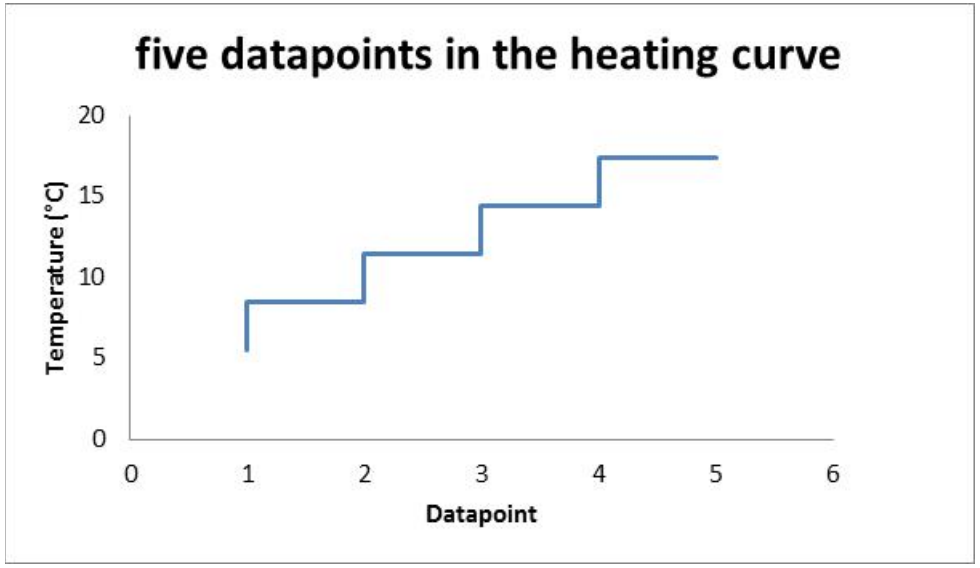


Heating inactivation in pan

Literature data	T (°C)	D (min)	log D
MURPHY et al., 2004.	55	45.87	1.66
	57.5	26.76	1.43
ground pork (40.2% fat)	60	5.07	0.71
	62.5	2.56	0.41
	65	1.91	0.28
	67.5	0.36	-0.44
	70	0.083	-1.08
JUNEJA et al., 2001	58	6.68	0.83
	60	6.65	0.83
Pork (8.5% fat)	62.5	1.62	0.21
	65	0.87	-0.06
JUNEJA et al., 2001	58	8.65	0.94
	60	5.48	0.74
Beef (12.5% fat)	62.5	1.5	0.18
	65	0.67	-0.17
SMITH et al., 2001.	55	9.05	0.96
	58	2.26	0.35
Low fat ground beef (4.8%)	61	0.57	-0.24
	64	0.15	-0.82
SMITH et al., 2001.	55	10.55	1.02
	58	2.15	0.33
Low fat ground beef (4.8%)	61	0.41	-0.39
	64	0.07	-1.15
SMITH et al., 2001.	55	10.27	1.01
	58	2.06	0.31
Low fat ground beef (4.8%)	61	0.43	-0.37
	64	0.14	-0.85
Velasquez et al. (2010)	55	3.846154	0.59
	58	0.653595	-0.18
Ground pork (2.5% fat)	60	0.263158	-0.58
	62	0.20284	-0.69
	63	0.119474	-0.92
JUNEJA et al., 2010	60	2.12766	0.33
Lean ground beef	65	0.364964	-0.44
	71	0.146628	-0.83





Heating inactivation in pan

Appendix B Observed data used to model heat inactivation of *Salmonella* during pan-frying.

Observed Batch	Start temperature[a] (°C)	Heating time (min)	End temperature (°C) per measured Meatball (MB)									
			MB 1	MB 2	MB 3	MB 4	MB 5	MB 6	MB 7	MB 8	MB 9	MB 10
2	5.1	7	19.9	23.5	31.9	34.2	39.1	39.5	39.8	42.7	44.5	56.3
3	5.1	7	18.2	18.3	20.1	20.6	27.0	28.3	29.5	41.0		
4	5.1	7	23.3	32.5	34.0	35.5	37.9	42.4	47.9	52.1	54.0	59.0
5	5.6	10	42.1	44.3	48.6	52.2	54.3	54.6				
6	7.2	10	37.2	46.0	49.1	50.1	62.2	66.8				
7	7.2	10	39.9	40.1	42.3	44.8	50	51.5				
8	8.4	6	32.1	33.1	39.1	53.2	55.2	59				
9	10.2	11	21.1	24.5	27.3	33.1	36.3	41.7	47			
10	10.2	5	45.1									

[a] Measured in the batter.

= 33 %

Model approach

Temperature profile:

$$T(t) = T_o + (T_{end} - T_o) / t$$

$$EF_{Tref} = \sum_{start}^{end} 10^{\frac{T(t)-Tref}{z}} \Delta t$$

$$LR_{pan\text{-}frying} = \frac{EF_{Tref}}{D_{Tref}}$$

Parameter values

T_o = 5-10° C

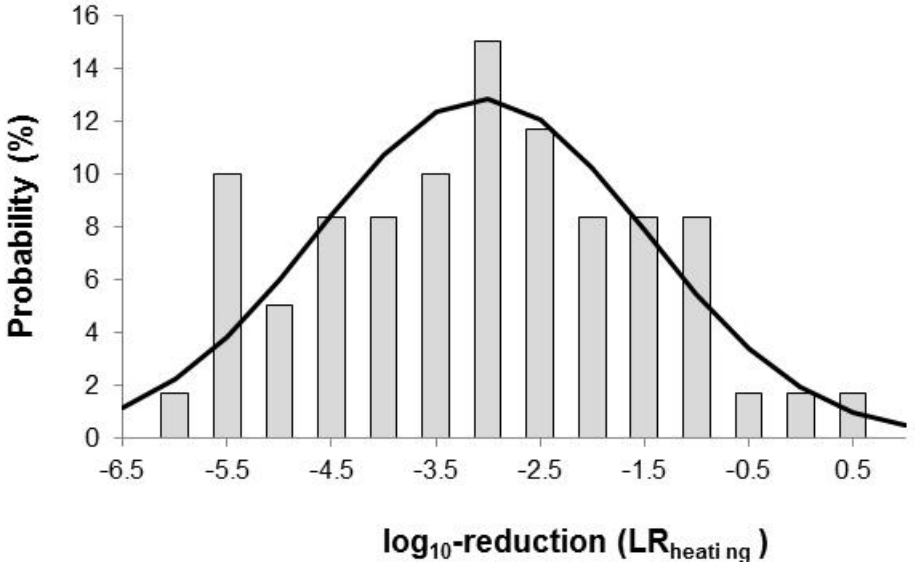
T_{end} = 18-67° C

t = 7-11 min

T_{ref} = 60° C

z = 7.34° C

D_{Tref} = 1.8 min



Risk of salmonellosis from consumption of “frikadeller”

Processing step	Basic activity	Source/model reference
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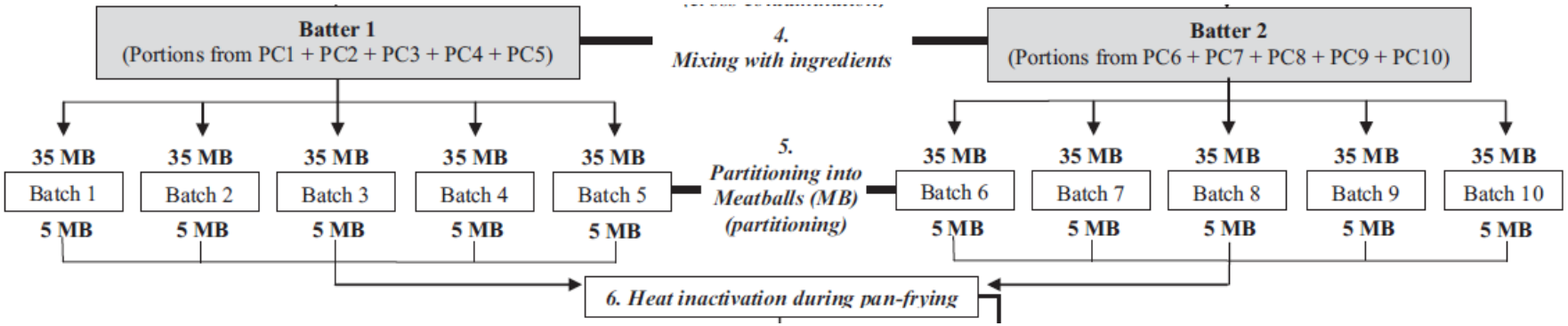
~ Poisson (10^(log10(HP$_{\text{conc crit area}}$) – 10^(HP$_{\text{log log reduction}}$)))



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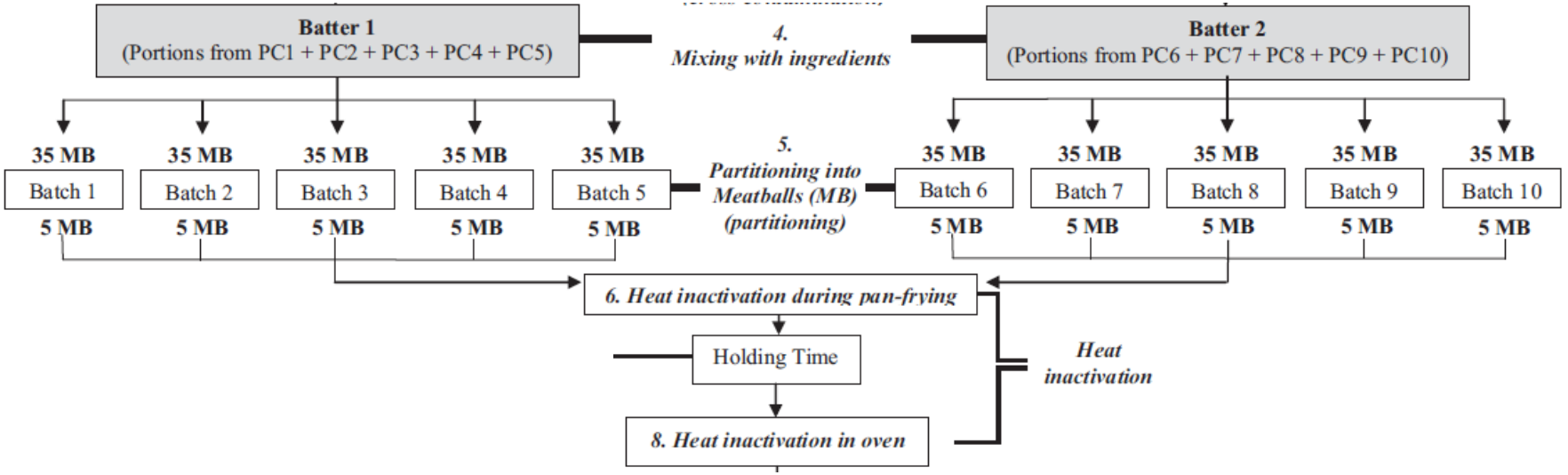
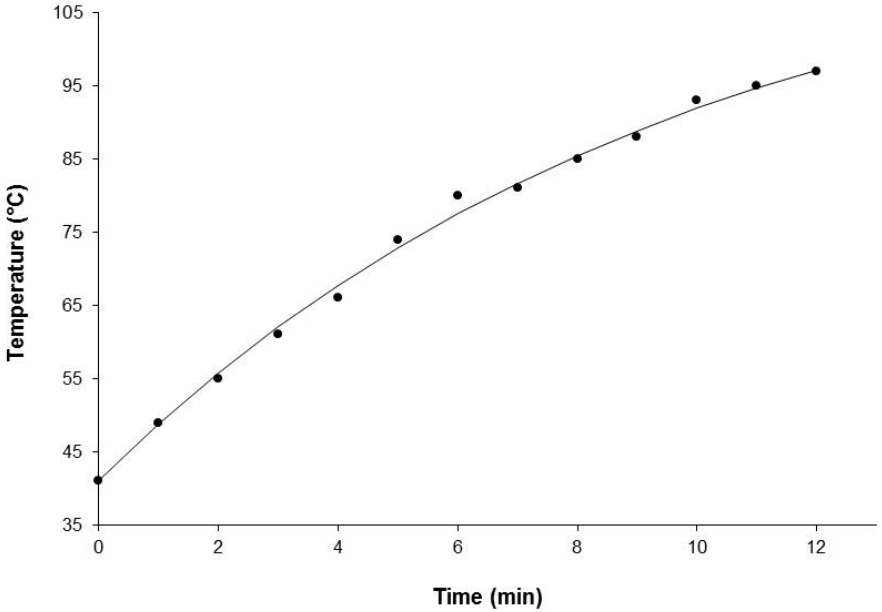
Heat inactivation in oven	8. inactivation	this study



Heating inactivation in oven



Observed (•) and fitted with Newton's Law equation (——) temperature profiles of heating of meatball in oven until an end temperature of 95° C.

Model approach	Parameter values
Temperature profile:	T_a = 121°C
$T(t) = T_a + (T_o - T_a) \cdot \exp(-k \cdot t)$	T_o = 25-45°C
	k = 6.1 h^{-1}
$EF_{Tref} = \Delta t$	*t* = 4-11 min
	T_{ref} = 65-75°C
$LR_{oven} = \frac{EF_{Tref}}{D_{Tref}}$	z = 7.34°C
	D_{Tref} = 0.4-0.02 min

Data on eight meatballs used to determine the degree of heat inactivation in oven

Observed meatball	Start temperature (T_o, ° C)[a]	Heating time (t, min)[b]	End temperature (° C)[c]	T_{ref} (° C)	Log (LR_{oven})[d]
1	25.4	11.1	89.8	95	3.74
		5.4	65.8	75	0.25
		5.2	64.6	74	0.07
		5.0	63.4	73	-0.10
		4.8	62.2	72	-0.27
		4.6	61.0	71	-0.44
		4.4	59.8	70	-0.61
		3.5	53.8	65	-1.46

Risk of salmonellosis from consumption of “frikadeller”

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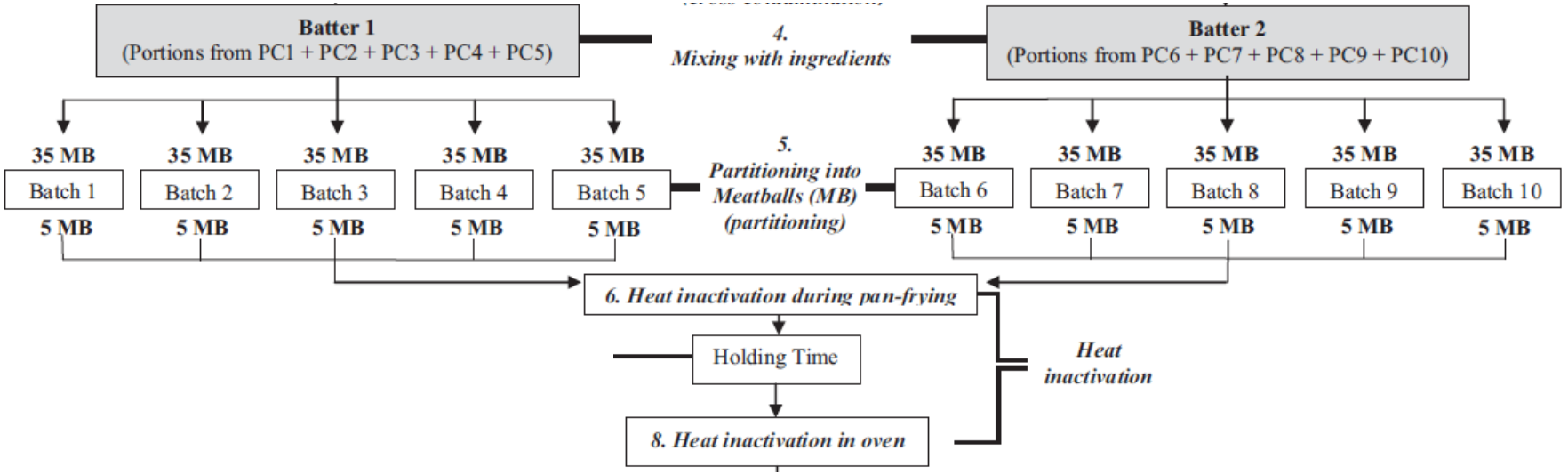
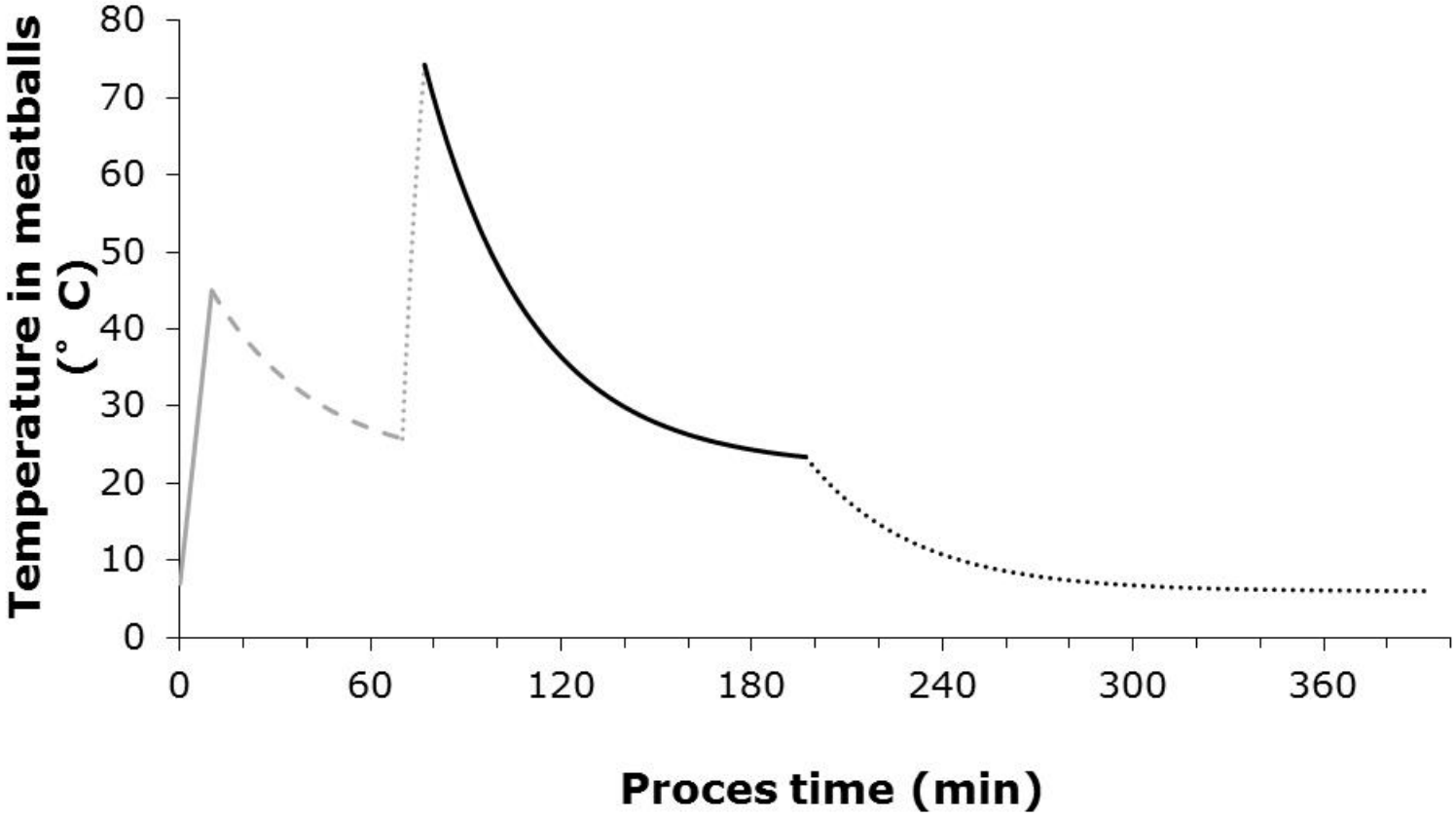
Heat inactivation in oven	8. inactivation	this study

~ Normal ($HO_{avg\ log\ log\ red}$, $HO_{st\ dev\ log\ log\ red}$)



Example of temperature profile for a meatball during processing at the catering sector



Involving: pan frying (grey full line), holding time (grey dashed line), heating in oven (grey dotted line), serving time (black full line), and chilled storage (black dotted line)

Risk of salmonellosis from consumption of “frikadeller”

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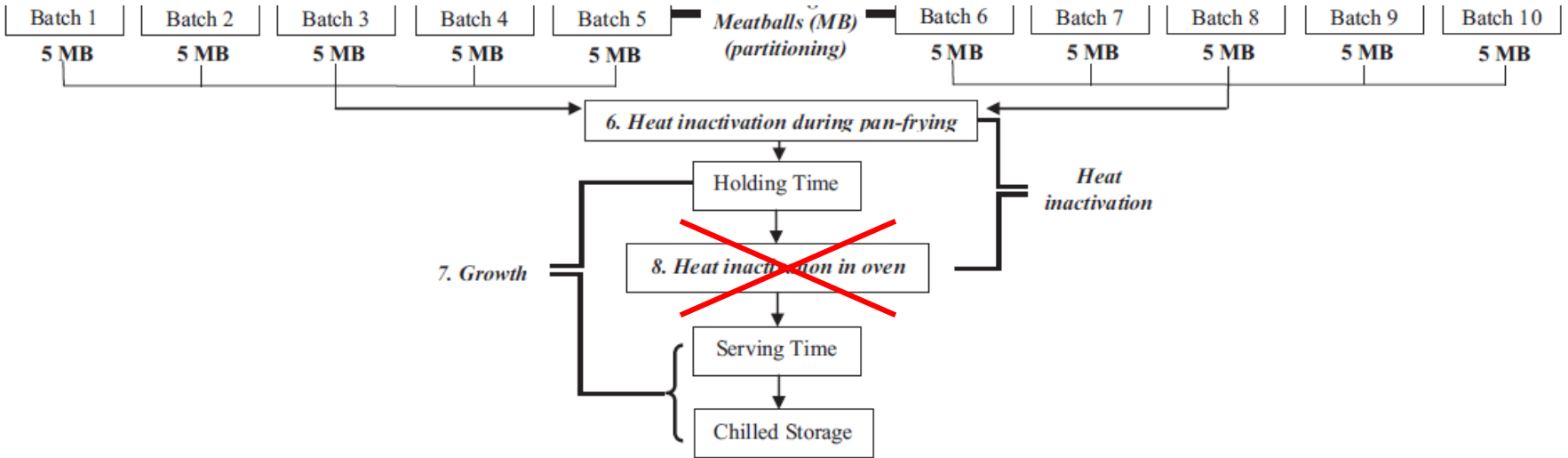
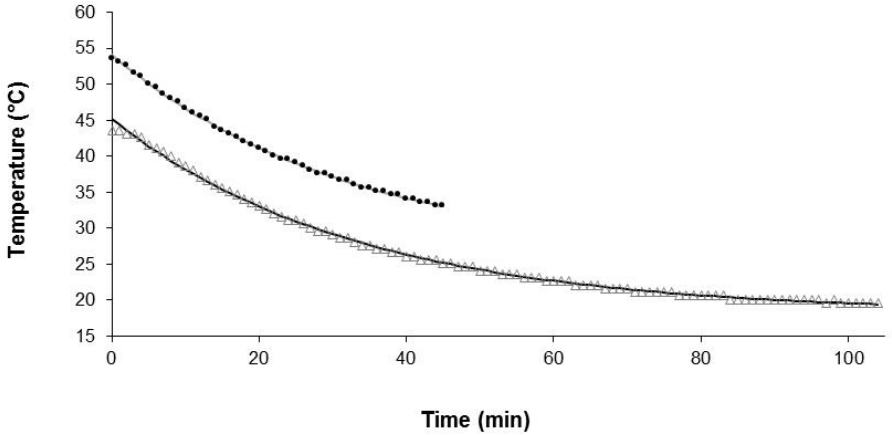
Serving time plus cold storage until 6° C is reached	7. growth	Møller et al. (2013), this study



Growth during storage



Observed (•, Δ) and fitted (grey line, black line) temperature profiles of the cooling ofmeatballs in kitchens one and two, respectively. Fitting done with Newton's Law resulting in k=1.8 h−1 in both catering units.

Model approach

Temperature profile:

$T(t) = T_a + (T_o - T_a) \cdot \exp(-k \cdot t)$

- T_a = 20-30°C
- T_o = 18.2-66.8°C
- k = 1.8 h^{-1}
- t = 0-90 + 120 + 210 min

Growth model:

$$\mu_{max} = (b \cdot (T - T_{min}) \cdot (1 - \exp(c \cdot (T - Tmax))))^2$$

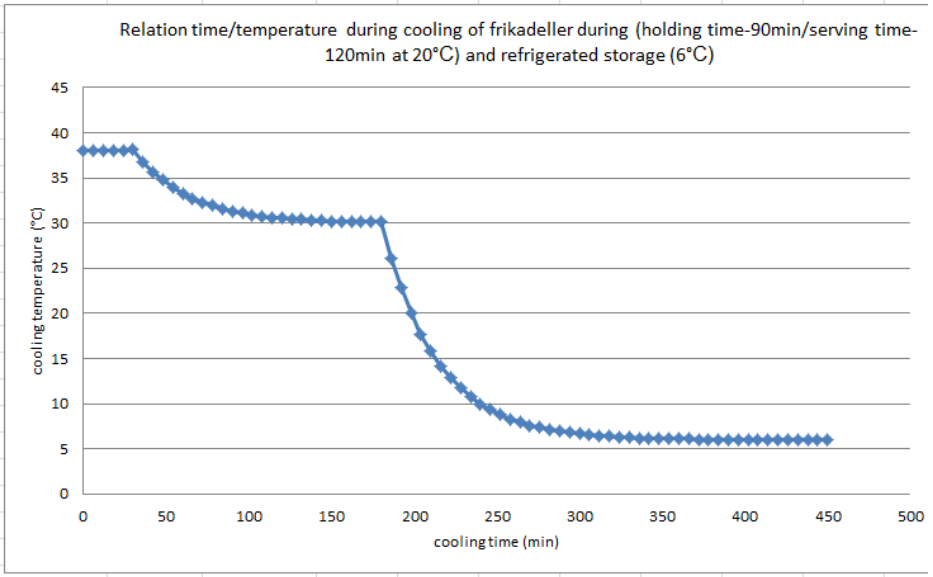
b = (0.04 $h^{-0.5} \cdot C^{-1}$) c = (0.43 C^{-1})

T is temperature T_{min} (3.94° C) T_{max} (48.0° C)

$$Lag\ time = RLT \cdot ln(2) / \mu_{max}$$

where *RLT* is the relative lag time of 3.10

	cooling (min)	Temperatur	µmax (1/h)	lag (h)	lag (min)	coolingtid minus la	0=ikke vækst ; 1=vækst
13	0	38	1.61246	1.3	80	80	0
14	6	38	1.61246	1.3	80	74	0
15	12	38	1.61246	1.3	80	68	0
16	18	38	1.61246	1.3	80	62	0
17	24	38	1.61246	1.3	80	56	0
18	30	38	1.62437	1.3	79	49	0
19	36	37	1.50509	1.4	86	50	0
20	42	36	1.40895	1.5	91	49	0
21	48	35	1.33107	1.6	97	49	0
22	54	34	1.26772	1.7	102	48	0
23	60	33	1.21599	1.8	106	46	0
24	66	33	1.17361	1.8	110	44	0
25	72	32	1.13879	1.9	113	41	0
26	78	32	1.11010	1.9	116	38	0
27	84	32	1.08642	2.0	118	34	0
28	90	31	1.06684	2.0	121	31	0
29	96	31	1.05062	2.0	123	27	0
30	102	31	1.03716	2.1	124	22	0
31	108	31	1.02599	2.1	125	17	0
32	114	31	1.01671	2.1	127	13	0
33	120	31	1.00899	2.1	128	8	0
34	126	30	1.00256	2.1	128	2	0
35	132	30	0.99720	2.2	129	-3	1
36	138	30	0.99274	2.2	130	-8	1
37	144	30	0.98903	2.2	130	-14	1
38	150	30	0.98593	2.2	131	-19	1



54 min. growth of Salmonella at 30C

6 min. growth of Salmonella at 26C

Growth during storage

Table 4. Change in log concentration Δlog C_x (T_o, RTK, $t_{holding}$) for different start temperatures (T_o) observed after pan-frying, for different room temperatures in the kitchen (RTK) and holding times $t_{holding}$ between 0 and 90 min followed by 120 min serving time and cold storage at 6°C.

RTK – Room Temperature in the Kitchen (°C)	T_o	Δlog C_x (T_o, RTK, $t_{holding}$=90 min)	Category[a]	Δlog C_x (T_o, RTK, $t_{holding}$=0 min)	Category
30	18.2	0.72	2	0.00	2
	32.0	0.96	2	0.24	2
	33.0	1.00	2	0.28	2
	40.2	1.13	2	0.41	2
	42.0	1.17	2	0.46	2
	43.0	1.18	2	0.46	2
	44.0	1.22	2	0.50	2
	45.0	1.22	2	0.50	2
	46.0	1.23	2	0.51	2
	47.0	1.18	2	0.46	2
	55.0	0.58	3[b]	-0.14	3
	56.0	0.17	3	-0.33	3
	57.0	-0.04	3	-0.64	3
	58.0	-0.26	3	-0.98	3
	59.0	-0.84	3	-1.57	3
	62.2	-3.64	3	-4.36	3
25	18.2	0.19	2	0.00	1
	32.0	0.40	2	0.00	1
	40.2	0.59	2	0.09	2
	46.0	0.64	2	0.17	2
	52.0	0.47	3[b]	-0.01	3
	55.0	0.14	3	-0.33	3
	57.0	-0.26	3	-0.74	3
	59.9	-1.04	3	-1.50	3
	62.2	-3.40	3	-3.85	3
20	18.2	0.00	1	0.00	1
	32.0	0.00	1	0.00	1
	35.5	0.02	2	0.00	1
	40.2	0.15	2	0.00	1
	46.0	0.19	2	0.00	1
	53.0	-0.01	3	-0.19	3[c]
	55.0	-0.22	3	-0.38	3
	57.0	-0.58	3	-0.74	3
	59.0	-1.27	3	-1.44	3
	62.2	-3.43	3	-3.58	3

[a] Process determining the result (see section 2.3.1.8). 1: lag phase; 2: growth; 3: inactivation.
[b] Both growth and inactivation can occur within a time temperature profile. The data point is assigned to the category where it gives the best fit to the linear model.
[c] No observations fall in category 2.

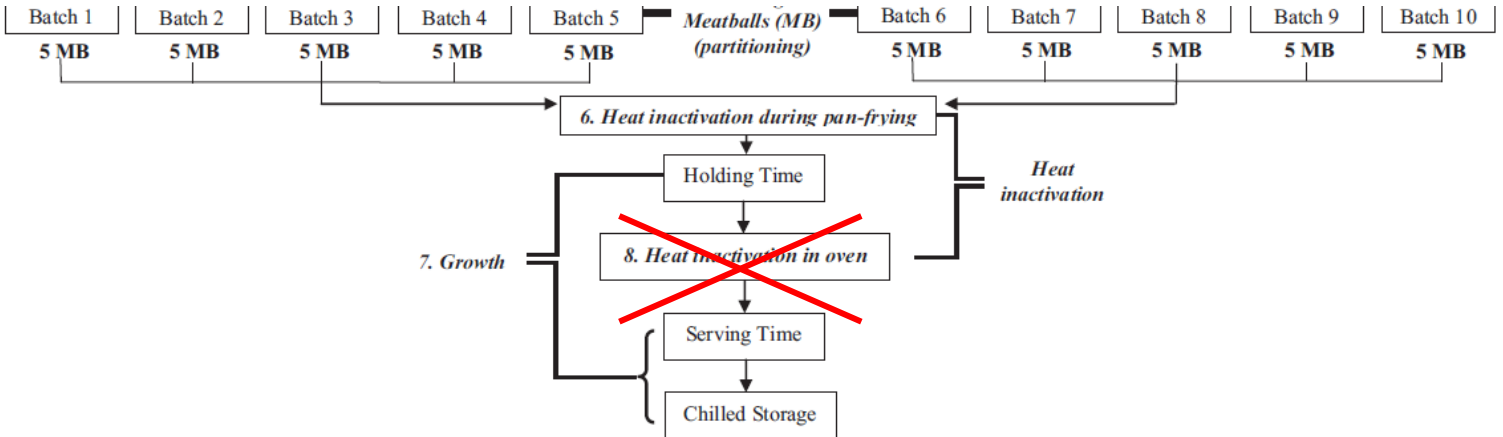
In the QMRA Monte Carlo Normal distribution of T_o:

- mean 40.3
- standard deviation 12

Risk of salmonellosis from consumption of “frikadeller”

Processing step	Basic activity	Source/model reference
Reception	1. initial contamination	Hansen et al. (2010)
Slicing	2. partitioning	Nauta (2005)
Grinding slices into portions	3. cross contamination	Møller et al. (2012)
Mixing of ingredients	4. mixing	Nauta (2005)
Dividing into meatballs	5. partitioning	Nauta (2005)
Heating inactivation in pan	6. inactivation	this study
Holding time	7. growth	Møller et al. (2013), this study
plus		
Heat inactivation in oven	8. inactivation	this study
Serving time plus cold storage until 6° C is reached	7. growth	Møller et al. (2013), this study

$$= (10\hat{}(\log10(HO_{conc\ after\ inac}) + 10\hat{}(\Delta\log C_x)))$$



2 QMRA framework

Risk of salmonellosis from consumption of "frikadeller"

Processing step	Basic activity	Source/model reference
Reception	1. initial contamination	Hansen et al. (2010)
Slicing	2. partitioning	Nauta (2005)
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Holding time	7. growth	Møller et al. (2013), this study
plus Heat inactivation in oven	8. inactivation	this study
Serving time plus cold storage until 6° C is reached	7. growth	Møller et al. (2013), this study
Estimation of the risk	9. dose response	FAO/WHO (2002)

$$Pill_{dose} \sim 1 - (1 + (C_{conc\ after\ \Delta log\ Cx} / \beta)^{\alpha}$$

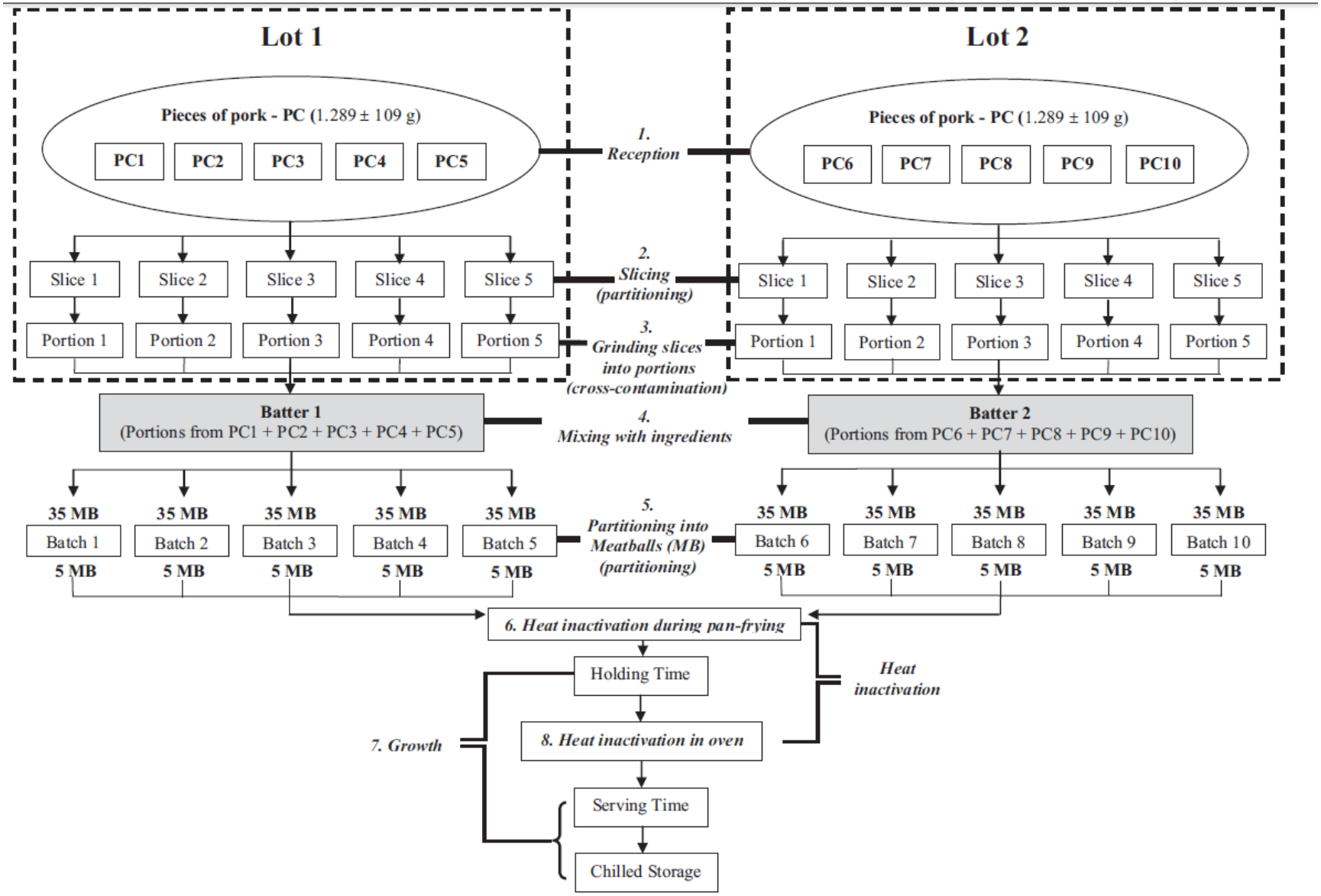
where $C_{conc\ after\ \Delta log\ Cx}$ is the concentration of *Salmonella* spp. in a meatball at the time of serving,

α was -0.1324 and

β equal to 51.45 (FAO/WHO, 2002).

The mean probability of illness from consumption of meatballs was used as the risk estimate

Risk of salmonellosis from consumption of 'frikadeller'



. 1. Schematic presentation of processing for one lot of Danish meatballs (MB) at a catering unit. Grey boxes indicate the mix of ingredients into two individual batches of batter.

Baseline model

Processing step	Parameters
Reception	Prevalence = 4.2% Concentration = 91 % 0.04-4 CFU/g 9 % → 400 CFU/g
Slicing	
Grinding slices into portions	
Mixing of ingredients	
Dividing into meatballs	
Heating inactivation in pan	
Holding time	
plus Heat inactivation in oven	75° C
Serving time plus cold storage until 6° C is reached	RTK = 20° C
Estimation of the risk	

6 QMRA modelling

Scenario analysis

- Evaluate processing practices
- Test control measures

Scenario **1A – 1G**

Holding time

↓

Heating in oven

core temperature:

75, 74, 73, 72, 71, 70 and 65° C

↓

Hot serving

Scenario 2

Baseline scenario

RTK = 20° C

Holding time

↓

Hot serving

Scenario **3A – 3C**

Holding time

↓

Hot serving

↓

Cold storage

Room **T**emp in the **K**itchen

(**RTK** = **20, 25 and 30° C**)

↓

Cold serving

6 QMRA modelling

Scenario analysis

Challenge 1:

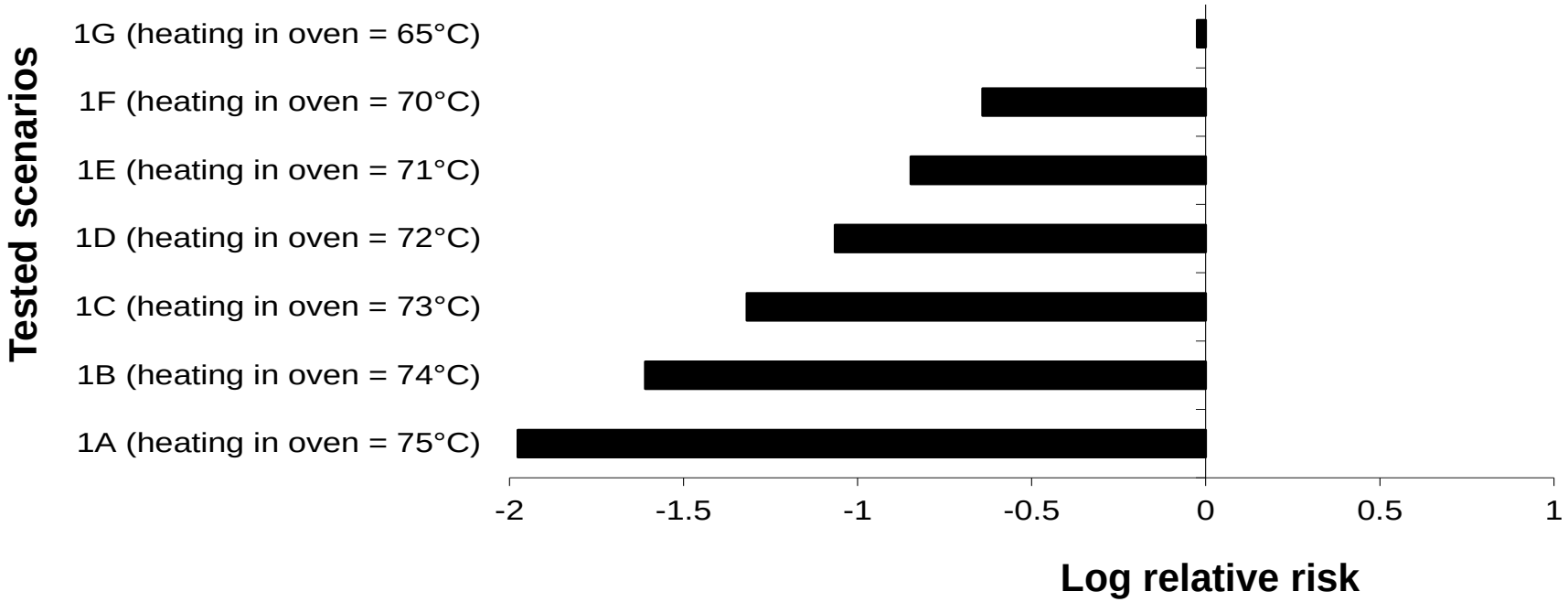
Are the recommendations of Food Safety Authorities good enough?

Core temperature of "frikadeller" in oven

1A	1B	1C	1D	1E	1F	1G
75° C	74° C	73° C	72° C	71° C	70° C	65° C

Baseline scenario: no oven, RTK = 20° C

(1 case in 10,000 servings)



6 QMRA modelling

Scenario analysis

Challenge 2:

Is it safe to consume cold “frikadeller”?

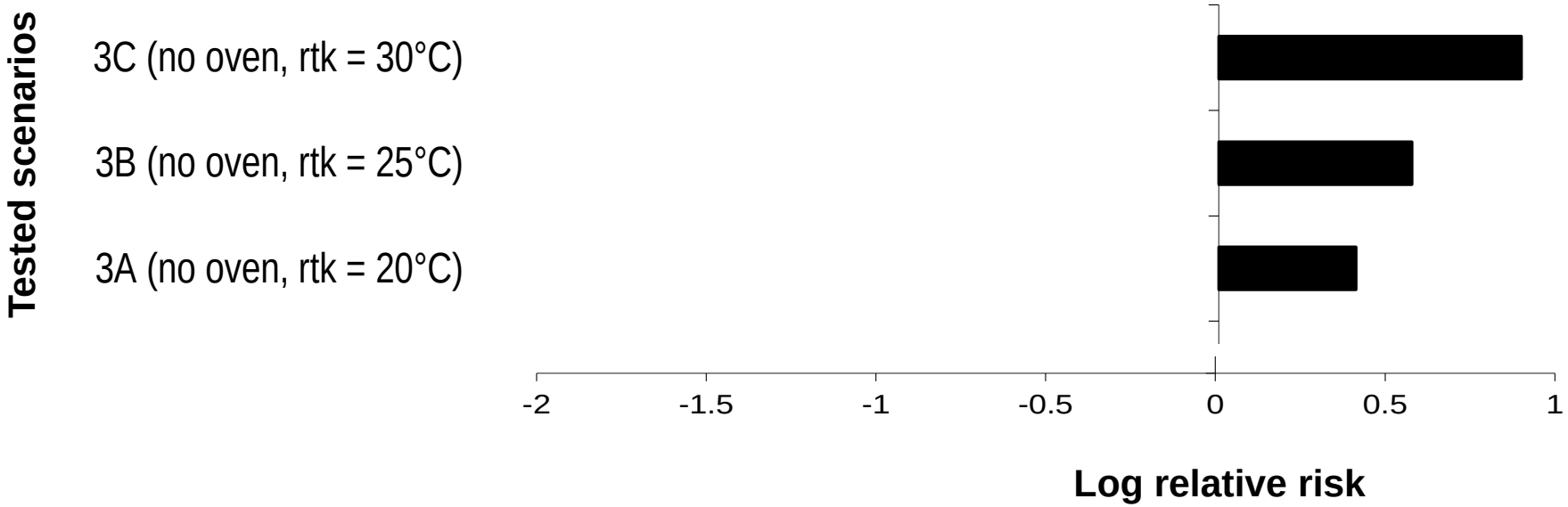
Holding time (0 to 90 min)
+
Serving time (120 min)
+
Refrigerated storage (210 min)

Room Temperature in the Kitchen (RTK)

3A	3B	3C
20° C	25° C	30° C

Baseline scenario: no oven, RTK = 20° C

(1 case in 10,000 servings)



6 QMRA modelling

Scenario analysis

Challenge 3:

How many people would became ill with salmonellosis in Denmark per year?

serving times/year =	**24**		Danish population (2013) =	5600000		20% of Danish population =	1120000	
1A (heating in oven = 75°C)	1B (heating in oven = 74°C)	1C (heating in oven = 73°C)	1D (heating in oven = 72°C)	1E (heating in oven = 71°C)	1F (heating in oven = 70°C)	1G (heating in oven = 65°C)	2 (no oven nor storage)	3C (no oven, rtk = 30°C)
0.000005	0.000012	0.000027	0.000044	0.000081	0.000122	0.000541	0.001217	0.003332
						5.41E-04	1.22E-03	3.33E-03
137	332	717	1186	2168	3291	14556	32706	89562

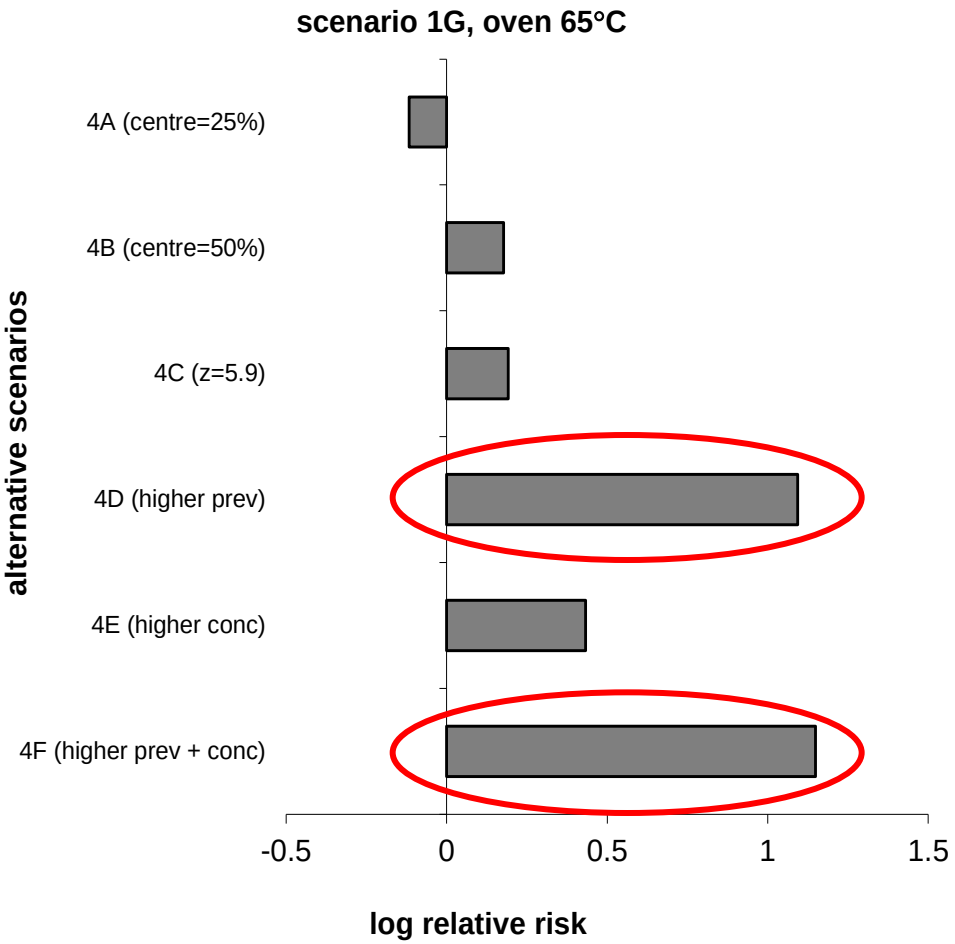
6 QMRA modelling

Sensitivity analysis

Investigated aspect	Baseline model	Sensitivity analysis	Alternative scenario
✓ Fraction of "frikadelle" with Survival of *Salmonella*	 = 33 %	= 25 %	4A
		= 50 %	4B
✓ Heating inactivation according to meat matrix	z-value = 7.34	z-value = 5.90	4C
✓ Prevalence of *Salmonella* in pork	4.2 %	20.0 %	4D
✓ Concentration of *Salmonella* in pork	91 % 0.04-4 CFU/g 9 % → 400 CFU/g	88 % 0.04-4 CFU/g *12 % 40-400 CFU/g*	4E
✓ ↑ prevalence and ↑ concentration			4F

6 QMRA modelling

Sensitivity analysis



↑ **impact on the risk estimates**

Combination of

↑ prevalence and ↑ concentration

Remarks and future perspectives

Risk assessment of *Salmonella* spp. in Danish meatballs produced at the catering sector

Cleide O. de A. Møller[1], Maarten J. Nauta[1], Donald W. Schaffner[2], Paw Dalgaard[3], Bjarke B. Christensen[1,4], Tina B. Hansen[1]

- ✓ The model flexible structure allows scenario analysis
- ✓ Core temperatures > 70° C inactivate *Salmonella*
- ✓ *No growth of Salmonella if:*
  - *RTK = < 20° C*
  - *For 3.5 h until refrigeration*
  - *Refrigerated storage = < 6° C*

To be investigated

- ✓ Heat inactivation :
  - Specific heating profile
  - "Frikadelle" batter
- ✓ Growth
  - Heat injured *Salmonella* cells
- ✓ Improvement of the previously developed models (transfer and growth)

Acknowledgements

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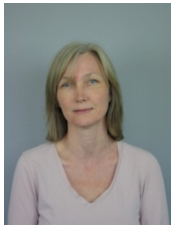
Tina Beck Hansen


Maarten Nauta


Donald W. Schaffner


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Bjarke Bak Christensen

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